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ORIGINAL RESEARCH

ANTIBACTERIAL ACTIVITY OF ANDROGRAPHOLIDE ON THE GROWTH OF PORPHYROMONAS GINGIVALIS

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ABSTRACT

***Introduction**: Porphyromonas gingivalis is the main bacteria that causes periodontitis, and the use of antibiotics such as metronidazole can cause side effects and resistance. Andrographolide from the bitter plant is a natural alternative that has potential as an antibacterial. **Aim**: This study was aimed to evaluate the antibacterial activity of Andrographolide against P. gingivalis. **Methods:** This study using the disc diffusion method at concentrations of 6 mg/mL, 8 mg/mL, and 10 mg/mL. Metronidazole 0.5 mg/mL was used as a positive control and 96% ethanol as a negative control. **Results:** Andrographolide concentrations of 6 mg/mL and 8 mg/mL produced a moderate inhibition zone, while a concentration of 10 mg/mL produced a strong inhibition zone. **Conclusion:** The potential effect of Andrographolide has antibacterial activity against P. gingivalis and shows an increase in inhibition with increasing concentration.*

***Keywords**: Andrographolide, Porphyromonas gingivalis, antibacterial, periodontitis.*

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INTRODUCTION

The periodontal tissues are the supporting structures of the teeth and consist of four main components: gingiva, cementum, periodontal ligament, and alveolar bone. These components function synergistically to support and stabilize the teeth within the jaw.[1] Damage to the periodontal tissues may result in a chronic inflammatory condition known as periodontitis, which can lead to serious consequences such as tooth mobility and, in advanced stages, tooth loss.[2]

The World Health Organization (WHO) has reported that approximately 3.7 billion people worldwide suffer from oral health problems. Periodontitis is one of the most prevalent oral diseases, ranking second after dental caries. According to WHO data, periodontitis has been detected in individuals as young as 15 years of age, with a prevalence of 60.2%, and reaches its highest prevalence among individuals aged 35– 44 years, at 77.2% globally. These data indicate that periodontal disease is not merely a local oral condition but represents a significant global public health concern requiring

special attention.[3]

In Indonesia, data from the 2018 Basic Health Research (RISKESDAS) showed that the highest prevalence of periodontitis occurred in individuals aged 45–54 years, with a prevalence rate of 77.8%. A decrease in prevalence was observed in individuals aged 65 years and older, at 66%. Periodontitis was slightly more prevalent in women than in men, with prevalence rates of 74.7% and 73.2%, respectively. Periodontitis is a destructive inflammatory disease that affects the tooth- supporting tissues and leads to progressive degradation of the periodontal ligament and alveolar bone. *Porphyromonas gingivalis* is recognized as a major etiological agent of periodontitis. This bacterium is a Gram-negative anaerobe with a unique ability to modulate the host immune response. *P. gingivalis* produces several virulence factors, including lipopolysaccharides and extracellular proteases (gingipains), which contribute to the destruction of the soft tissues surrounding the teeth.[4,5]

Conventional periodontal therapy, such as scaling and root planing, has been proven effective in the management of periodontitis. However, these procedures often fail to completely eliminate pathogenic bacteria within the oral cavity, particularly those organized in biofilms. To enhance treatment outcomes, adjunctive therapies are commonly employed, including local antiseptics such as chlorhexidine or systemic antibiotics like metronidazole. Nevertheless, the uncontrolled use of antibiotics in the community poses a significant risk of promoting bacterial resistance.[6]

Sambiloto (*Andrographis paniculata*) is a medicinal herbal plant widely distributed across Asia, including India, Sri Lanka, Pakistan, and Indonesia, and is also extensively cultivated in regions such as Mauritius, East and West Indies, China, and Thailand.[7] This plant is well known for its antibacterial activity against a broad range of pathogenic microorganisms. The principal bioactive compound in *A. paniculata* is andrographolide, which is present in all parts of the plant, with the highest concentration found in the leaves, ranging from 2.5% to 4.8% of the dry weight.[8] Andrographolide has an extremely bitter taste and is highly soluble in methanol, ethanol, pyridine, acetic acid, and acetone, while exhibiting low solubility in water and ether.[9]

Previous studies have demonstrated the antibacterial activity of andrographolide against *Porphyromonas gingivalis*. Sanita Ayu reported the largest inhibition zone at a concentration of 5 mg/mL, although the antibacterial activity was classified as weak. Banerjee et al. also reported that andrographolide inhibited bacterial growth with different MIC values for Gram-negative and Gram-positive bacteria. However, the concentration-dependent antibacterial activity of andrographolide against *P. gingivalis* has not been fully elucidated. Therefore, this study aimed to evaluate the antibacterial activity of andrographolide against *P. gingivalis* at different concentrations using the agar diffusion method..[10]

METHODS

This study was an in vitro laboratory-based experimental study employing a posttest-only control group design. The experimental design consisted of several groups receiving different treatments, and observations were conducted

after the treatments were applied. The primary objective of this study was to evaluate the antibacterial activity of andrographolide by visually assessing the formation of inhibition zones.

The test microorganism used in this study was *Porphyromonas gingivalis* ATCC 33277, obtained from the Microbiology Laboratory of Universitas Andalas. The study included several treatment groups consisting of andrographolide at concentrations of 6 mg/mL, 8 mg/mL, and 10 mg/mL, as well as two control groups: a positive control using metronidazole at a concentration of 0.5 mg/mL and a negative control using 96% ethanol. Based on calculations using Federer's formula, each treatment was performed in five replicates, resulting in a total of 25 samples.

The inclusion criteria were *P. gingivalis* ATCC 33277 cultures that had been freshly subcultured and exhibited active growth on culture media, as well as sterilized Mueller–Hinton Agar (MHA). The exclusion criteria included *P. gingivalis* cultures contaminated with other bacteria or fungi and andrographolide solutions contaminated by environmental factors. The independent variable in this study was andrographolide at concentrations of 6 mg/mL, 8 mg/mL, and 10 mg/mL, while the dependent variable was the growth of *P. gingivalis* ATCC 33277, assessed based on the diameter of the inhibition zones formed.

This study was conducted from October to November 2025 at the Microbiology Laboratory of Universitas Andalas. Prior to the commencement of the experiment, ethical and administrative approval was obtained from the Faculty of Dentistry, Universitas Baiturrahmah.

The experimental procedure began with bacterial rejuvenation to obtain fresh and active cultures. One loopful of the stock culture was aseptically inoculated onto slanted Mueller–Hinton Agar using a zigzag streaking technique near a Bunsen burner and incubated in an anaerobic jar at 37°C for 24 hours. A bacterial suspension was then prepared by transferring 2–3 loopfuls of colonies into 5 mL of sterile 0.9% NaCl solution, homogenized using a vortex mixer for 10 seconds, and adjusted to match the turbidity of a 0.5 McFarland standard.

The antibacterial activity was assessed using the disc diffusion method on Mueller–Hinton Agar. A volume of 0.1 mL of the *P. gingivalis* suspension was evenly spread onto five Petri dishes using a sterile cotton swab and allowed to stand for 5 minutes. Each Petri dish contained five paper discs: three discs impregnated with andrographolide at concentrations of 6 mg/mL, 8 mg/mL, and 10 mg/mL; one disc containing 96% ethanol as the negative control; and one disc containing metronidazole 0.5 mg/mL as the positive control. All Petri dishes were incubated for 24 hours.

The inhibition zones formed around each disc were measured using a digital caliper. Both vertical and horizontal diameters were recorded. For oval-shaped inhibition zones, the average diameter was calculated by summing the longest and shortest diameters and dividing the total by two.

The collected data were analyzed bivariately using SPSS software. Data normality was assessed using the Shapiro–Wilk test to determine whether the data followed a normal distribution. Homogeneity of variances was

evaluated using Levene's test. When the assumptions of normality. and homogeneity were satisfied, the data were further analyzed using one-way analysis of variance (One-Way ANOVA) to determine differences in mean values among the treatment groups.

RESULTS

Table 1 presents the mean diameters of the inhibition zones produced by andrographolide against *Porphyromonas gingivalis* at concentrations of 6 mg/mL, 8 mg/mL, and 10 mg/mL, along with the positive control (metronidazole) and the negative control (ethanol).

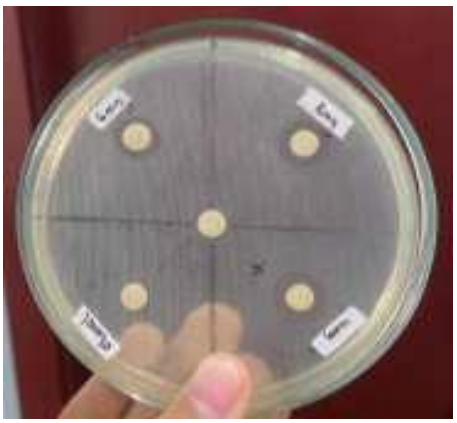

Figure 1. Inhibition zones produced by different consentrations of andrographolide against P. gingivalis

Table 1. Inhibition zone diameters of andrographolide against *Porphyromonas gingivalis* growth

Treatment	Repetition					Mean (mm)	Category
	1	2	3	4	5		
6 mg/ml	8,50	9,50	7,25	6,00	6,00	7,45	Moderate
8 mg/ml	12,25	11,25	9,75	7,25	7,25	9,55	Moderate
10 mg/ml	9,75	11,25	9,25	10,00	11,25	10,30	Strong
Metronidazole (+)	9,25	9,75	10,25	11,75	11,50	10,50	Strong
Ethanol (-)	0	0	0	0	0	0	No inhibition

The results showed that andrographolide at concentrations of 6 mg/mL and 8 mg/mL produced mean inhibition zone diameters of 7.45 mm and 9.55 mm, respectively, which were classified as moderate antibacterial activity. At a concentration of 10 mg/mL, the inhibition zone diameter increased to 10.30 mm, indicating strong antibacterial activity. These findings demonstrate a concentration- dependent increase in the inhibitory effect of andrographolide, with higher concentrations resulting in larger inhibition zones and enhanced antibacterial activity.

For comparison, the positive control metronidazole produced an inhibition zone of 10.50 mm, which was also categorized as strong activity. In contrast, the negative control (ethanol) did not produce any inhibition zone (0 mm), indicating the absence of antibacterial activity against *P. gingivalis*.

The observational data were subsequently subjected to a normality test. The Shapiro–Wilk test was applied because the sample size was fewer than 50. The data were considered to be normally distributed when the significance value (p) was greater than 0.05. Based on this analysis, the following results were obtained.

Table 2. Shapiro-Wilk Test

Groups	p-value
6 mg/ml	0,403
8 mg/ml	0,329
10 mg/ml	0,254
Metronidazole (+)	0,494

Based on the results presented in Table 2, all treatment groups showed p-values greater than 0.05. Therefore, it can be concluded that the data were normally distributed and met the normality assumption required for subsequent parametric statistical analyses.

Table 3. Levene's Test

Variable	p-value	α
Porphyromonas gingivalis inhibition zone	0,080	0,050

The homogeneity test showed a p-value of 0.080 ($p > 0.05$), indicating homogeneous variances across the groups. Since both the normality and homogeneity assumptions were satisfied, a one-way analysis of variance (One-Way ANOVA) was performed to assess differences in mean values among the treatment groups.

Table 4. One-Way Anova Test

Variable	p-value	α	Decision
Porphyromonas gingivalis inhibition zone	0,025	0,05	Ha accepted

As shown in Table 4, the significance value was 0.025 ($p < 0.05$), indicating a statistically significant difference in the mean diameters of the inhibition zones among the treatment groups. Therefore, the null hypothesis (H_0) was rejected, and the alternative hypothesis (H_a) was accepted.

DISCUSSION

The results of this study demonstrated that all tested concentrations of andrographolide exhibited inhibitory activity against *Porphyromonas gingivalis*. Andrographolide at concentrations of 6 mg/mL and 8 mg/mL showed moderate antibacterial activity, whereas the 10 mg/mL concentration exhibited strong antibacterial activity. These findings indicate that the antibacterial effect of andrographolide increases with higher concentrations, suggesting a concentration- dependent inhibitory effect. This result is consistent with the findings reported by Pandey et al., who demonstrated that extracts of *Andrographis paniculata* leaves and stems showed enhanced antibacterial activity with increasing concentrations. The highest inhibition zones were observed at 100% concentration against *Pseudomonas aeruginosa* and *Escherichia coli*, while the lowest inhibition zones occurred at 25% concentration, Further supporting the concentration-dependent nature of the antibacterial activity.[11]

Andrographolide is the primary bioactive compound in *A. paniculata* and is widely recognized for its antibacterial properties. A study conducted by Banerjee et al. further supports the role of andrographolide as an effective antibacterial agent. Their findings showed that andrographolide inhibited the growth of Gram-positive bacteria such as *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 100 µg/mL and exhibited bacteriostatic activity. Additionally, andrographolide demonstrated effectiveness against Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The antibacterial mechanism of andrographolide is primarily associated with the inhibition of DNA synthesis, leading to disruption of RNA and protein synthesis and ultimately preventing bacterial cell proliferation, while bacterial cell wall formation remains unaffected.[10]

The findings of this study indicate that andrographolide exhibits antibacterial activity against *Porphyromonas gingivalis* at all tested concentrations. Moderate inhibitory effects were observed at concentrations of 6 mg/mL and 8 mg/mL, while a strong antibacterial effect was demonstrated at 10 mg/mL. These results suggest that the antibacterial activity of andrographolide increases proportionally with concentration, indicating a concentration- dependent effect.

This observation is consistent with previous studies reported by Pandey et al., which showed that leaf and stem extracts of *Andrographis paniculata* displayed enhanced antibacterial activity as the concentration increased. In their study, the largest inhibition zones were observed at a 100% concentration against *Pseudomonas aeruginosa* and *Escherichia coli*, whereas the smallest inhibition zones were found at a 25% concentration, further supporting the concentration-dependent nature of the antibacterial effect.[12,13]

Andrographolide is the principal bioactive compound of *A. paniculata* and has been extensively reported to possess antibacterial properties. Evidence from a study by Banerjee et al. supports its effectiveness as an antibacterial agent. Their results demonstrated that andrographolide inhibited the growth of Gram-positive bacteria such as *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 100 µg/mL and exhibited bacteriostatic activity. Moreover, andrographolide was shown to be effective against several Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The antibacterial mechanism of andrographolide is primarily attributed to the inhibition of DNA synthesis, which subsequently disrupts RNA and protein synthesis, thereby preventing bacterial proliferation without affecting bacterial cell wall formation.[14,15]

CONCLUSION

Andrographolide demonstrated anti-bacterial activity against *P. gingivalis*, with inhibitory effects increasing in a concentration-dependent manner, indicating its potential as an antibacterial agent.

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